# Cyclobutane Thymidine Dimers are Present in Human Urine Following Sun Exposure: Quantitation Using $^{32}$P-Postlabeling and High-Performance Liquid Chromatography

Frank Le Curieux and Kari Hemminki

Department of Biosciences at Novum, Karolinska Institute, Huddinge, Sweden

**Cyclobutane thymidine dimer (T=T) is the major DNA photoproduct formed in human skin after solar radiation. We have developed a $^{32}$P-postlabeling method suitable for quantitating T=T in human urine with a detection limit of about 0.5 fmol per 10 µl urine. The method was used in the present study to measure the daily T=T urinary level of two volunteers over a 15 d period, including frequent sun exposures ranging from 0 to 5 h daily. T=T was not detected before or immediately (4 h) after the initial sun exposure but was first observed in urine samples collected 18 h after the initial exposure. Thereafter, urinary T=T levels gradually increased up to a peak reached about 3 d after the maximum sun exposure. The levels decreased during the following days but were still detectable 8 d after the last sun exposure. About 70–75% decrease in excreted T=T was observed after 8 d. The T=T levels measured in urine were lower but in the same order of magnitude as the levels expected after a theoretical calculation based on previous published results and reasonable assumptions. This study shows the occurrence of cyclobutane thymidine dimers in human urine after skin exposure to solar radiation.** ***Key words: $^{32}$P-postlabeling/creatinine/cyclobutane thymidine dimers/high-performance liquid chromatography/human urine/photoproduct/sun exposure/ultraviolet radiation. J Invest Dermatol 117:263–268, 2001***

Solar radiation is harmful to human skin and it induces sunburn, photoaging, and cancer. Ultraviolet (UV) radiation represents only 5% of the intensity of the sun emission energy reaching the earth's surface but it is the main cause of skin damage. About 95% of UV radiation reaching the earth's surface is UVA (315–400 nm) and 5% is UVB (280–315 nm), whereas UVC (100–280 nm) is totally filtered by the atmospheric ozone layer (IARC, 1992). Both UVA and UVB have deleterious effects on human skin cells, but UVB is believed to be the main contributor to erythema, i.e., skin reddening (Augustin *et al*, 1997), and skin cancer (Black *et al*, 1997; English *et al*, 1997). UVB induces specific DNA damages termed photoproducts: cyclobutane dimers and 6-4 photoproducts between neighboring pyrimidine bases on the same DNA strand (Bykov and Hemminki, 1995). *In situ* studies performed on human skin biopsies following UVB or solar-simulated radiation helped to improve the general understanding of the formation and repair of photoproduct in skin DNA, and the influence of parameters such as skin type, age, gender, and use of sun screen (Bykov *et al*, 1998a, 1998b, 1999; 2000; Hemminki *et al*, 1999; Xu *et al*, 2000a, b); however, the collection of skin biopsies is tedious because it is a clinical invasive procedure.

Nucleotide excision repair is a DNA repair system in which specific enzymes remove a 24–29 base DNA fragment containing a lesion. After excision, further enzymatic processing breaks down the oligomer to nucleotides, nucleosides and bases that are released in the circulation and finally reutilized or excreted in urine (Diem, 1962; Försti *et al*, 1988). Several lesions of endogenous or dietary origin have been detected in urine. Oxidative DNA damage such as thymidine glycol and 8-hydroxy-deoxyguanosine have been measured in urine using high performance liquid chromatography (HPLC)/electrochemical detection and gas chromatography/mass spectrometry (Simic, 1992). DNA alkylation products such as 3-methyl-adenosine and 3-ethyl-adenosine have been quantitated in urine by gas chromatography/mass spectrometry or enzyme-linked immunosorbent assay (Shuker *et al*, 1993). The analysis of DNA lesions in urine generally involves prepurification steps using normal- or reversed-phase column, or immunoaffinity columns.

The aim of this study was to develop a noninvasive method able to quantitate (or reflect reliably) the formation and the removal of photoproducts in human DNA, and demonstrate its usefulness. We focused on human urine and looked for one of the major photoproducts formed after solar-simulated radiation, i.e., cyclobutane thymidine dimer (T=T) (Bykov *et al*, 1998a, 1999). Preliminary results have been published elsewhere (Hemminki *et al*, 1999, 2001). Cyclobutane thymidine dimers have been previously measured in the urine of psoriasis patients treated with psoralen and UVA, using an enzyme-linked immunosorbent assay antibody method (Ahmad *et al*, 1999; Cooke *et al*, 2001).

## MATERIALS AND METHODS

**Principle** Cyclobutane thymidine dimer is not an appropriate substrate for T4 polynucleotide kinase and, consequently, cannot be labeled directly. As the parent dinucleotide (TpT) is easily labeled, we chose to convert T=T to TpT by irradiation with UVC at 254 nm. The source

Manuscript received June 27, 2000; revised March 30, 2001; accepted for publication April 2, 2001.

Reprint requests to: Dr. Kari Hemminki, Department of Biosciences at Novum, Karolinska Institute, 141 57 Huddinge, Sweden. Email: kari.hemminki@cnt.ki.se

Abbreviations: T=T, cyclobutane thymidine dimer; TpT, parent thymidine dimer.

of UVC irradiation was a Stratalinker UV Crosslinker 2400 with lamps providing almost monochromatic 254 nm light. This lamp was used both to prepare the reference T=T, and to revert T=T to TpT. The reversion was carried out using a dose of 10 kJ per $m^2$. The reversion rate ranged from 30 to 50% and was determined in each experiment by irradiating three standards (12, 36, and 108 fmol T=T dissolved in 40 μl mQ water) and using the $^{32}$pTpT peak area obtained to draw a calibration curve.

**Sample collection** Two volunteers (one man, 32 y old, 78 kg; one woman, 30 y old, 60 kg) provided urine samples collected twice a day (first morning urine, and evening urine) during 15 d. Each volunteer provided one additional sample on day 23. Urine samples were recovered in 2-ml Eppendorf tubes, labeled with the date and time of collection, immediately frozen at –20°C and kept frozen until analysis.

**Sun exposure** The sample collection took place at the end of February–beginning of March. On day 0 of the study, the untanned volunteers (skin types IV and V, according to IARC, 1992) traveled from Sweden (cloudy winter weather with no significant sun exposure) to the Caribbean where they were exposed to solar radiation. The first sun exposure took place on day 1 in the afternoon. The sun exposures mainly took place during periods spent at the beach. Approximately one fourth of the time was spent under direct exposure during active periods (swimming or sport), the remaining three-fourths being spent lying partially protected by palm tree shadow. No prolonged sun bathing was performed. Sunscreen (protecting factor 6, about 1 μl per $cm^2$ applied, Diffey, 1996) was applied on the legs, arms, and shoulders from days 1 to 9 for the male volunteer, and from days 1 to 15 for the female volunteer. All sun exposures and their duration were recorded daily. Knowing the height and weight of each volunteer, we could evaluate the body surface at 2.1 $m^2$ for the man, and 1.7 $m^2$ for the woman (Diem, 1962). We considered that half of the body surface was exposed to sun when only arms and legs were uncovered (volunteers wearing shorts and T-shirt).

During the 2 wk period, the weather was rather sunny but the sky always displayed some scattered light clouds especially along the coastal line (typical of the islands of the Caribbean archipelago). It is known that even a complete light cloud cover does not stop more than 50% of UVB energy – compared with that from clear sky – from reaching the earth's surface (Diffey, 1991). The island of the study is situated at 16° of latitude, so the typical value for ambient UVB radiation can be estimated at about 6000 minimal erythema doses per year using the radiation level of Hawaii, situated at 20° of latitude, as a comparison (Diffey, 1991).

**Sample analysis** Urine samples were allowed to melt at room temperature, filtrated through 0.22 μm filters and 10 μl was injected on the UV–HPLC system. In the system used, the retention time for T=T was 16 min whereas the parent TpT had a retention time of 30 min, so they did not coelute. The fraction eluting between 15.5 and 17 min was collected, freeze-dried, and dissolved in 40 μl mQ water. The mixture was then submitted to UVC irradiation (10 kJ per $m^2$) for reversion of T=T to TpT. TpT was labeled on the 5′ side using the protocol described previously (Bykov and Hemminki, 1995). The labeled samples were diluted with water up to 12 μl and injected on the radioisotope HPLC system.

**Chromatographic systems** UV-HPLC analyses were performed on a Beckman instrument (model 126 pump) operated with System Gold and coupled to a model 168 diode-array detector (Beckman Instruments, San Ramon, CA). The urine samples were chromatographed on a 5 μm, 4.6 × 250 mm reversed phase C18 Luna column from Phenomenex (Genetec, Kungsbacka, Sweden). A precolumn filter was positioned before the column. The column was eluted isocratically for 5 min with 50 mM ammonium formate buffer (pH 4.6) and then with a gradient from 0 to 30% methanol in 45 min at a flow rate of 0.7 ml per min.

The labeled samples were analyzed on a Beckman instrument (model 126 pump) operated with System Gold and coupled to a model 171 radioisotope detector (Beckman Instruments). The urine samples were chromatographed on a C18 Luna column (5 μm, 2 × 250 mm) from Phenomenex, connected to a C18 Kromasil precolumn (5 μm, 2 × 50 mm) also from Phenomenex, and to a precolumn filter. The column was eluted isocratically for 3 min with the buffer (500 mM ammonium formate, 20 mM orthophosphoric acid, pH 4.6), and then with a gradient from 0 to 20% methanol in 30 min. During all the analysis, the Luna column was maintained at 40°C using a Cool Pocket manufactured by Keystone Scientific (Belle fonte, PA). A switch system was placed between the precolumn and the analytical column. During the first 2.5 min, the switch was on the waste position and the eluate coming out of the precolumn, containing mainly unreacted $^{32}$P isotope, was directed to the waste container. At 2.5 min, the switch was turned to the column position to reconnect the precolumn to the analytical column.

**Data analysis** For each urine sample the T=T level was adjusted with the creatinine level, which reflects kidney glomerular filtration and is nearly constant in the urine of a given individual. Creatinine levels were determined by UV absorbance at 230 nm, i.e., area of the peak corresponding to creatinine in UV–HPLC chromatograms. Fifteen of the 58 samples were analyzed in triplicate and the standard deviation was usually lower than 10% of the mean (data not shown). Other samples were analyzed once.

**Amount of T=T "expected" in urine** The amount of cyclobutane thymidine dimer that can theoretically be expected in urine was previously evaluated to 15 fmol per μl urine (Hemminki *et al*, 2001). The theoretical calculation was based on the following assumptions: (i) a dose of 400 J per $m^2$ of solar simulating radiation (1–2 minimal erythemal dose, about 15–30 min sun in Stockholm area in summer) induces the formation of six TT=T/$10^6$ nucleotides in skin DNA, giving a total amount of dimer (AT=T, GT=T, CT=T, and TT=T) at 24 T=T/$10^6$ nucleotides; (ii) the amount of DNA extracted from a 4 mm diameter skin biopsy is about 10 μg, thus the amounts expected in 1 $m^2$ of skin are 800 mg of DNA and 60 nmol of T=T; (iii) 25% of T=T is removed from skin DNA in the first 24 h after UV radiation (Xu *et al*, 2000b); (iv) all T=T removed from skin DNA ends up in the urine (1 litre per 24 h). The possible skin desquamation resulting from frequent exposure was not taken into account in this calculation. This parameter probably contributes to a loss of T=T.

## RESULTS

**Hypothesis testing** Our search for photoproducts in urine postulates that T=T released by DNA repair are not degraded further and are excreted in urine as a dimer. The assumption was tested by incubating at 37°C cyclobutane thymidine dimers with $S_9$ mixture, which contains enzymes from rat liver homogenate along with appropriate buffers and cofactors. After 24 h, the reaction mixture still contained unchanged amounts of unmodified cyclobutane thymidine dimers suggesting that the hypothesis was correct.

**Radioisotope chromatogram** A typical radioisotope chromatogram obtained for a urine sample after sun exposure containing a small amount (about 3 fmol) of T=T is presented on **Fig 1**. The peak appearing at 27 min was identified as $^{32}$pTpT, i.e., $^{32}$P-labeled TpT, on the basis of three parameters: (i) it displayed the same retention time as the standard $^{32}$pTpT; (ii) a cochromatography experiment showed that it coeluted with the standard $^{32}$pTpT; (iii) it demonstrated the same photochemical

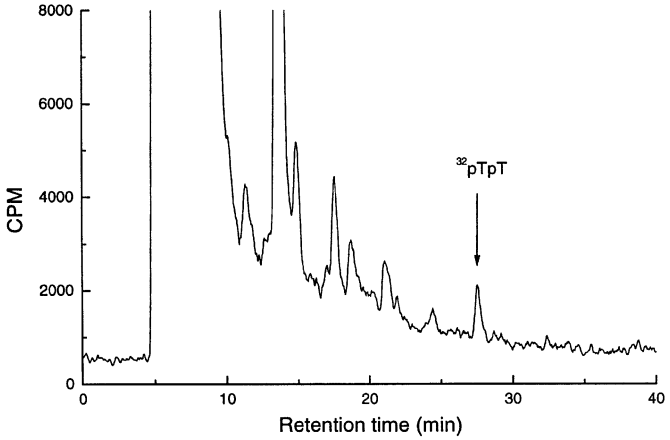


**Figure 1. Radioisotope HPLC chromatogram of human urine containing a small amount (≈ 3 fmol) of T=T photoproduct.** The *arrow* shows the elution of the labeled TpT, the conversion product of T=T.

**Table I. Urinary cyclobutane thymidine dimer (T=T) levels for male volunteer**

| | | T=T levels | | | | |
|---|---|---|---|---|---|---|
| | | fmol per μmol creatinine | | | | |
| Day | Sun exposure (h×m$^2$) | Morning | Evening | Mean | fmol per μmol creatinine per m$^2$ body surface | μmol per day[a] |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 4.2 | 0 | 0 | 0 | 0 | 0 |
| 2 | 1.6 | 21 | 59 | 40 | 19 | 0.6 |
| 3 | 4.2 | 75 | 164 | 120 | 57 | 1.9 |
| 4 | 8.4 | 213 | 243 | 228 | 108 | 3.6 |
| 5 | 0 | 513 | 342 | 427 | 204 | 6.7 |
| 6 | 1.1 | 677 | 407 | 542 | 258 | 8.5 |
| 7 | 2.1 | 635 | 624 | 629 | 300 | 9.8 |
| 8 | 6.3 | 586 | ND | 586 | 279 | 9.1 |
| 9 | 3.2 | ND | 560 | 560 | 267 | 8.7 |
| 10 | 0 | 514 | 397 | 455 | 217 | 7.1 |
| 11 | 0 | 628 | 291 | 460 | 219 | 7.2 |
| 12 | 1.1 | ND | 524 | 524 | 249 | 8.2 |
| 13 | 0 | 393 | 339 | 366 | 174 | 5.7 |
| 14 | 3.2 | 447 | 344 | 396 | 188 | 6.2 |
| 15 | 1.1 | 389 | 158 | 274 | 130 | 4.3 |
| … | | | | | | |
| 23 | 0 | ND | 64 | 64 | 30 | 1.0 |

[a]Calculation based on an average value of 200 μmol creatinine per kg per day for men. ND, not determined.

**Table II. Urinary cyclobutane thymidine dimer (T=T) levels for female volunteer**

| | | T=T levels | | | | |
|---|---|---|---|---|---|---|
| | | fmol per μmol creatinine | | | | |
| Day | Sun exposure (h×m$^2$) | Morning | Evening | Mean | fmol per μmol creatinine per m$^2$ body surface | μmol per day[a] |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 3.4 | 0 | 0 | 0 | 0 | 0 |
| 2 | 1.3 | 68 | 116 | 92 | 54 | 0.9 |
| 3 | 3.4 | 117 | 136 | 127 | 75 | 1.2 |
| 4 | 6.8 | 275 | 178 | 227 | 133 | 2.2 |
| 5 | 0 | 279 | 304 | 291 | 171 | 2.8 |
| 6 | 0.9 | 359 | 321 | 340 | 200 | 3.3 |
| 7 | 1.7 | 476 | 289 | 383 | 225 | 3.7 |
| 8 | 8.5 | Nd | ND | ND | ND | ND |
| 9 | 2.6 | ND | 399 | 399 | 235 | 3.8 |
| 10 | 0 | 613 | 344 | 479 | 282 | 4.6 |
| 11 | 0 | 934 | 738 | 836 | 492 | 8.0 |
| 12 | 0 | ND | 616 | 616 | 362 | 5.9 |
| 13 | 0 | 644 | 352 | 498 | 293 | 4.8 |
| 14 | 2.6 | 454 | 310 | 382 | 225 | 3.7 |
| 15 | 0.9 | 201 | ND | 201 | 118 | 1.9 |
| … | | | | | | |
| 23 | 0 | ND | 62 | 62 | 36 | 0.6 |

[a]Calculation based on an average value of 160 μmol creatinine per kg per day for women. ND, not determined.

reactivity as the standard $^{32}$pTpT, i.e., UVC irradiation of $^{32}$pTpT produced labeled T=T and 6-4 photoproducts. Moreover, we confirmed that the $^{32}$pTpT peak was really representing UVC-reversed T=T and not TpT contamination. We spiked aliquots of T=T-free urine with known amounts of T=T. The radioisotope chromatogram showed no peak at 27 min for the control sample (T=T-free urine alone), the spiked samples showed a peak at 27 min, which was proportional to the amount of T=T added.

**Urinary T=T levels** The results on daily urinary dimer levels are presented in **Tables I** and **II**. A few values are missing in **Table I** and **II** due to the lack of samples. Before any significant sun exposure, the urine samples of both volunteers contained no T=T (the detection limit of our method is about 0.5 fmol per 10 μl urine). Cyclobutane thymidine dimer was not detected in the urine samples collected on day 1 in the morning or in the evening (4 h after the initial sun exposure). For both volunteers, T=T was first observed in the samples collected on day 2 in the morning, i.e., 18 h after the initial exposure. T=T urinary level gradually increased until a maximum on day 7 for the man and on day 11 for the woman. The highest individual T=T levels observed were 142 fmol per 10 μl urine for the man and 155 fmol per 10 μl urine for the woman, which correspond to 635 fmol per μmol creatinine and 934 fmol per μmol creatinine, respectively. For the man, T=T levels generally decreased after day 7, although a lower maximum appeared on day 12 and a shoulder was observed on day 14. For the

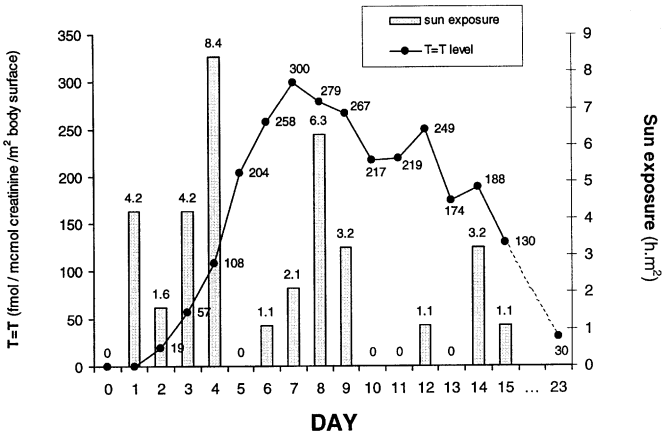


**Figure 2. Relation between sun exposure and urinary T=T level in male volunteer.**

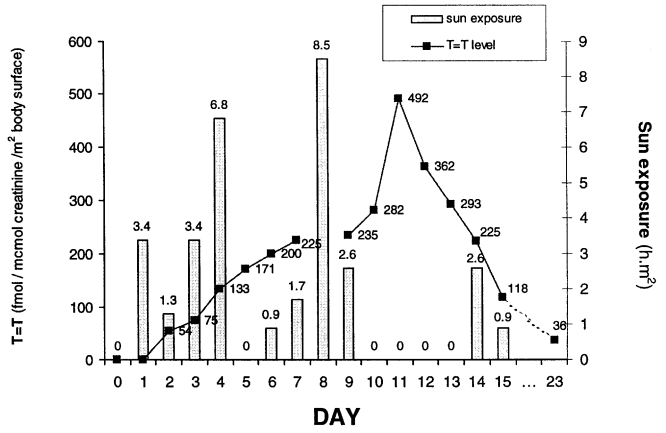


**Figure 3. Relation between sun exposure and urinary T=T level in female volunteer.**

**Table III. Trend analysis in the initial days: cumulative sun exposure *vs* daily urinary T=T level**

| | Male | | Female | |
|---|---|---|---|---|
| Day | Cumulative exposure (h per $m^2$) | Daily urinary T=T (fmol per μmol creatinine) | Cumulative exposure (h per $m^2$) | Daily urinary T=T (fmol per μmol creatinine) |
| 0 | 0 | 0 | 0 | 0 |
| 1 | 4.2 | 0 | 3.4 | 0 |
| 2 | 5.8 | 40[a] | 4.7 | 92[b] |
| 3 | 10 | 120[a] | 8.1 | 127[b] |
| 4 | 18.4 | 228[a] | 14.9 | 227[b] |

[a]Regression analysis: $R^2 = 0.95$; $p < 0.01$.
[b]Regression analysis: $R^2 = 0.92$; $p < 0.01$.

woman, T=T amount steadily decreased after day 12. On day 23, 8 d after the last significant sun exposure, urinary T=T amounts remained at detectable levels (12 fmol per 10 μl urine for the man, 4 fmol per 10 μl urine for the woman).

**Table III** presents the cumulative sun exposure and the daily urinary T=T level for the first 4 d. A regression analysis of the values shows a positive correlation between the cumulative sun exposure and the excreted T=T concentrations, both for the man and the woman. The data show a highly significant relationship between exposure and excreted levels during the first days of exposure.

In spite of the creatinine adjustment, the urinary T=T level in the morning remained generally higher than that in the evening, except for the first 3–4 d. During the first days, evening creatinine-corrected T=T levels were higher or similar to morning levels. To ease the comparison of the results between the man and the woman, that have different body surfaces, the T=T urinary levels were expressed in fmol per μmol creatinine per $m^2$ of body surface. These final values are plotted on **Figs 2** and **3**.

## DISCUSSION

This study is a sequential analysis on two individuals, and the results obtained should not be generalized before a larger population is studied.

The basic assumption of our work was that cyclobutane thymidine dimer is excreted unchanged to urine. Our test with microsomal enzymes suggested that no degradation took place. It had been shown earlier that a dinucleotide cross-link by cis-platinum is excreted to over 70% to urine when administered i.p. to rats (Försti *et al*, 1988).

**No urinary T=T before sun exposure** The absence of detectable T=T in urine collected before any significant sun exposure is obviously due to the absence or the very low level of T=T in skin DNA. The fact that T=T was not detected in the urine sample collected 4 h after the initial sun exposure but was observed 18 h after sun exposure shows that there is a latency period between the excision of T=T photoproducts from DNA and the appearance of T=T in urine. Indeed, during nucleotide excision repair, a 24–29 oligomer containing T=T is removed from the DNA. The postexcision processing that releases T=T as a monophosphate nucleotide dimer is certainly a partial reason for the delay. Moreover, T=T must be transferred from the cell in the epidermis to the lymphatic system and/or the blood before being filtrated through the kidney and finally released in the urine. The transfer of T=T from one body compartment to another is probably a slow step in the process because cell membranes are expected to be poorly permeable to a charged molecule such as T=T. No previously published suitable data was available to compare with the less than 18 h delay between T=T formation in DNA and its appearance in urine. Sontag *et al* (1995), however, reported that in mouse, T=T-containing DNA in cells were observed in lymph nodes as early as 1 h after 1.5 kJ per $m^2$ UV radiation exposure.

**Increase in T=T urinary levels after sun exposure** The gradual increase in T=T observed in the urine of both volunteers was related to the accumulation of photoproducts in skin DNA during the first days of exposure. The amount of T=T excised from DNA was consequently increased and, after transport and filtration delay, it was ultimately reflected as an increased level in urinary cyclobutane thymidine dimer. Maximal levels were reached when the rates of formation and removal were equally high. It was reported that in skin cells from hairless mice, irradiated for 11 consecutive days with a daily dose of 1500 J per $m^2$ UVB, cyclobutane thymine dimers accumulated in epidermal cells and reached a maximum level after 3 d. Thereafter, dimer levels dropped to a lower, more constant level (Vink *et al*, 1993). In the study of Försti *et al* (1988), rats were administered i.p. either cis-platinum dinucleotide adduct or DNA containing cis-platinum dinucleotide adduct. When the adduct was administered as such, 30% was recovered in urine after the 4 d, most of it (18%) being recovered during the first 24 h. When DNA containing the cis-platinum adduct was administered, only about 1% was recovered after 4 d, and the highest urinary amount was observed on the third day following administration (Försti *et al*, 1988). The difference in the time taken by cis-platinum adduct to reach the urine suggests

that there is a major delay associated with the removal of dinucleotide cross-link adduct from DNA and its release as a dimer.

**Three day latency period** In our study, there was a trend for a 3 d latency time between the maximum sun exposure and the maximum T=T level in urine. The urinary T=T maximum did not appear on the same day for the man and the woman (day 7 and day 11, respectively). This can be due to differences in the timetable of exposure (the highest exposure was on day 4 for the man and on day 8 for the woman) or differences in the rate of repair. The second T=T maximum observed for the man on day 12 can be associated with a prolonged sun exposure on day 8 followed by a lower exposure on day 9. One interpretation of the small shoulder appearing on day 14 for the man (but not for the woman) is that urinary T=T levels accurately follows UV-induced T=T levels in DNA. The shoulder seems indeed to reflect the 1 h sun exposure received by the man (and not the woman) on day 12.

T=T urinary levels observed on days 5–7 (following a period when both volunteers received the same number of hours of sun) were higher for the man than for the woman. Assuming that T=T urinary levels reliably reflect T=T DNA levels, this finding is not consistent with the fact that the man has a darker skin type than the woman (skin type V for the man and IV for the woman). The man should, therefore, be prone to lower levels of photoproducts formed in DNA than the woman, as melanin protects the genomic DNA from UV radiation. Our result can be explained by differences between the two volunteers in terms of: (i) repair efficiency (the man's excision repair system might release more free T=T than the woman's), or (ii) recovery of T=T in urine (the proportion of free T=T recovered in urine might be higher for the man than for the woman). Another explanation can also be a difference in the use of sunscreen (body surface treated, amount applied per $cm^2$) between volunteers.

The urinary T=T levels measured on day 23 showed that 8 d after the last exposure, the excretion level decreased by 77% for the man, and 69% for the woman. This is consistent with the higher urinary T=T levels observed for the man during the first week of the study.

**Morning–evening difference in T=T urinary levels** The difference between morning and evening creatinine-adjusted T=T levels tends to demonstrate that one or several of the processes involved (i.e., T=T excision from DNA, transport from cells to the lymphatic system or blood, kidney glomerular filtration rate) are more efficient during the night compared with during the late afternoon or early evening. An alternative explanation is that DNA damage accumulated during the daytime and, considering the delay in excretion, the levels were higher at night than during the subsequent day. This circadian variation can also be explained by a modification the glomerular filtration rate of cyclobutane thymidine dimer compared with that of creatinine, i.e., a relative higher filtration rate of T=T during the night and the early morning. Although a study on 10 healthy volunteers reported a decreased excretion of creatinine during the night and morning hours (Araki *et al*, 1983), we did not notice significant differences between morning and evening creatinine levels (data not shown). As expected, considering the higher body and muscle weight for the man, he displayed higher creatinine urinary levels than the female volunteer (mean value of 0.163 and 0.123 μmol per 10 μl, respectively, data not shown).

**Measured and expected T=T urinary levels in the same order of magnitude** Taking into account all the urine samples displaying detectable T=T levels, i.e., from day 2 of the study, the average T=T level in urine can be roughly evaluated to 382 and 371 fmol per μmol creatinine for the man and woman, respectively. Using the average creatinine level in urine cited above, we evaluated the average urinary T=T level to be 6.2 and 4.6 fmol per μl urine for the man and the woman, respectively. These values are in the same order of magnitude as the theoretically expected level of 15 fmol per μl urine (Hemminki *et al*, 2001).

**Skin type** This study detected T=T in the urine of individuals with rather dark skin (skin type IV and V). We can reasonably expect similar or higher urinary T=T levels for persons with fair skin (type I or II). For a given UV exposure, skin type II produces more UV-induced DNA damages than skin type V (Hemminki *et al*, 2001) and DNA repair rate does not differ significantly between different skin types (Xu *et al*, 2000b; Hemminki *et al*, 2001).

## CONCLUSIONS

In summary, this study used a new sensitive $^{32}$P-postlabeling method to demonstrate that cyclobutane thymidine dimers are present in the urine of two volunteers following extensive exposure to solar radiation. This study shows the occurrence of photoproducts in urine after skin exposure to solar radiation. The T=T levels measured in urine are in the same order of magnitude as the levels expected from a previous theoretical calculation. Future studies are needed to measure the sensitivity of the $^{32}$P-postlabeling method for quantitating urinary T=T following different levels of controlled solar UV exposure. We also plan to investigate the stability and the distribution of T=T in human body compartments (blood plasma) and its recovery in urine.

*The work was supported by the Swedish Cancer Fund and the Swedish Radiation Protection 1nstitute.*

## REFERENCES

Ahmad J, Cooke MS, Hussieni A, *et al*: Urinary thymine dimers and 8-oxo-2′-deoxyguanosine in psoriasis. *FEBS Lett* 46:549–553, 1999

Araki S, Murata K, Yokoyama K, Yanagihara S, Niinuma Y, Yamamoto R, Ishihara N: Circadian rhythms in the urinary excretion of metals and organic substances in "healthy" men. *Arch Environ Health* 38:360–366, 1983

Augustin C, Collombel C, Damour O: Measurements of the protective effect of topically applied sunscreens using in vitro three-dimensional dermal and skin equivalents. *Photochem Photobiol* 66:853–859, 1997

Black HS, deGruijl FR, Forbes PD, *et al*: Photocarcinogenesis: an overview. *J Photochem Photobiol B* 40:29–47, 1997

Bykov VJ, Hemminki K: UV-induced photoproducts in human skin explants analysed by TLC and HPLC-radioactivity detection. *Carcinogenesis* 16:3015–3009, 1995

Bykov VJ, Janssen C, Hemminki K: High levels of dipyrimidine dimers are induced in human skin by solar-simulating UV radiation. *Cancer Epidemiol Biomarkers Prev* 7:199–202, 1998a

Bykov VJ, Marcusson JA, Hemminki K: Ultraviolet B-induced DNA damage in human skin and its modulation by a sunscreen. *Cancer Res* 58:2961–2964, 1998b

Bykov VJ, Sheehan JM, Hemminki K, Young AR: In situ repair of cyclobutane pyrimidine dimers and 6-4 photoproducts in human skin exposed to solar simulating radiation. *J Invest Dermatol* 112:326–331, 1999

Bykov VJ, Marcusson JA, Hemminki K: Effect of constitutional pigmentation on ultraviolet B-induced DNA damage in fair-skinned people. *J Invest Dermatol* 114:40–43, 2000

Cooke MS, Evans MD, Burd RM, Patel K, Barnard A, Lunec J, Hutchinson PE: Induction and excretion of ultraviolet-induced 8-oxo-2′-deoxyguanosine and thymidine dimers in vivo: implications for psoralen and ultraviolet A. *J Invest Dermatol* 116:281–285, 2001

Diffey BL: Solar ultraviolet radiation effects on biological systems. *Phys Med Biol* 36:299–328, 1991

Diffey BL: Sunscreens, suntans and skin cancer. People do not apply enough sunscreen for protection. *Br Med J* 313:942, 1996

Diem K (ed): *Documenta Geigy, Scientific Tables*, 6th edn. Basle: Geigy JR, 1962, p 632

English D, Armstrong B, Kricker A, Fleming C: Sunlight and cancer. *Cancer Causes Control* 8:271–283, 1997

Försti A, Takala M, Laatikainen R, Hemminki K: Excretion kinetics of the DNA adducts of cis-diamminedichloroplatinum (II) formed in vitro in rat urine. *Carcinogenesis* 9:1745–1748, 1988

Hemminki K, Bykov VJ, Marcusson JA: Re: Sunscreen use and duration of sun exposure: a double-blind, randomized trial. *J Natl Cancer Inst* 91:2046–2047, 1999

Hemminki K, Xu G, Le Curieux F: *Ultraviolet Radiation-Induced Photoproducts in Human Skin DNA as Biomarkers of Damage and its Repair*. IARC Science Publication no. 154. Lyon: IARC, 2001, pp 69–79

*IARC*: *Monographs on the Evaluation of Carcinogenic Risks to Humans Solar and Ultraviolet Radiation*. IARC Science Publication no. 55. Lyon: IARC, 1992, pp 43–72

Shuker DE, Prevost V, Friesen MD, Lin D, Ohshima H, Bartsch H: Urinary markers for measuring exposure to endogenous and exogenous alkylating agents and precursors. *Environ Health Perspect* 99:33–37, 1993

Simic MG: Urinary biomarkers and the rate of DNA damage in carcinogenesis and anticarcinogenesis. *Mutat Res* 267:277–290, 1992

Sontag Y, Guikers CL, Vink AA, *et al*: Cells with UV-specific DNA damage are present in murine lymph nodes after in vivo UV irradiation. *J Invest Dermatol* 104:734–738, 1995

Vink AA, Berg RJ, de Gruijl FR, Lohman PH, Roza L, Baan RA: Detection of thymine dimers in suprabasal and basal cells of chronically UV-B exposed hairless mice. *J Invest Dermatol* 100:795–799, 1993

Xu G, Snellman E, Bykov V, Jansen C, Hemminki K: Cutaneous malignant melanoma patients have normal repair kinetics of UV-induced DNA damage in skin in situ. *J Invest Dermatol* 114:628–631, 2000a

Xu G, Snellman E, Bykov V, Jansen C, Hemminki K: Effect of age on the formation and repair of UV photoproducts in human skin in situ. *Mutat Res* 459:195–202, 2000b